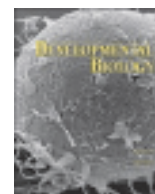


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Review

Epiblast morphogenesis before gastrulation

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ABSTRACT

The epiblast is a single cell-layered epithelium which generates through gastrulation all tissues in an amniote embryo proper. Specification of the epiblast as a cell lineage in early development is coupled with that of the trophoblast and hypoblast, two lineages dedicated to forming extramebryonic tissues. The complex relationship between molecular specification and morphogenetic segregation of these three lineages is not well understood. In this review I will compare the ontogeny of epithelial epiblast in different amniote groups and emphasize the diversity in cell biological mechanisms employed by each group to reach this conserved epithelial structure as the pre-requisite for gastrulation. The limitations of associating cell fate with cell shape and position will also be discussed. In most amniote groups, bi-potential precursors for the epiblast and hypoblast, similar to the inner cell mass in the eutherian mammals, are not associated with an apolar, inside location in the blastocyst. Conversely, a blastocyst cell with epithelial morphology and superficial location is not indicative of its trophoblast fate. The polar trophoblast is absent in all amniotes except for the eutherian mammals. In the avian, reptilian and eutherian groups, epithelialization of the epiblast occurs after its fate specification and involves a mesenchymal-to-epithelial transition (MET) process, whereas in the monotremes and marsupials, pre-epiblast cells adopt an epithelial morphology prior to their commitment to the epiblast fate. The conservation of an epithelialized epiblast is viewed as an adaptation to evolutionary constraints placed on pre-gastrulation ectoderm in the ancestral amniote. The relationship between epiblast MET and epiblast pluripotency will also be discussed. Whether such an MET/epithelialization process is advantageous for the self-renewal and/or differentiation of human epiblast stem cells *in vitro* is unclear.

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Introduction

The amniotes are a monophyletic clade of vertebrate animals consisting of three living groups: the mammals, reptiles and birds. The reptiles and birds, together with the prototherian mammals (monotremes), share many early developmental features, whereas the early embryogenesis in the metatherian (marsupial) and eutherian (placental) mammals are highly derived due to yolk reduction and an emphasis on placentation for nutritional supply. All amniote species, however, have an extraembryonic tissue organization that is evolutionarily conserved (Ferner and Mess, 2011; Sheng and Foley, 2012) and its genesis underlies key morphogenetic processes during their early development. Future intraembryonic tissues originate from a part of the early amniote embryo called the epiblast, a single cell-layered epithelial sheet which is the starting material for gastrulation. Morphogenesis leading to the formation of this epithelial epiblast shows diverse strategies used in different amniote groups to reach the earliest phylogenetically-conserved developmental stage. Understanding epiblast

morphogenesis in model organisms serves as a basis for unraveling its molecular regulation. Yet caution is needed when extrapolating species specific features and mechanisms to other amniotes. In this review I will provide a brief overview of major modes of epiblast formation in the amniotes. Emphasis will be given to several features in the mouse model which are not universally conserved, including the location of the epiblast precursor cells, the association of trophoblast fate with the earliest epithelial structure in the blastocyst, and the relationship between the polar trophoblast and the inner cell mass (ICM). I will also underscore the difference between epiblast fate specification and epiblast epithelialization (the process of collective polarization of epiblast-fated cells to form an epithelium) (Nakaya and Sheng, 2013), and emphasize the importance of the MET process accompanying the final step of epithelial epiblast formation in several amniote groups.

Epiblast formation in the mouse

Early development in the mouse has been discussed in detail in several recent reviews (Artus and Chazaud, 2014; Posfai et al., 2014; Schrode et al., 2013; Stephenson et al., 2012). The following summary is based on these reviews and on a recent paper describing the

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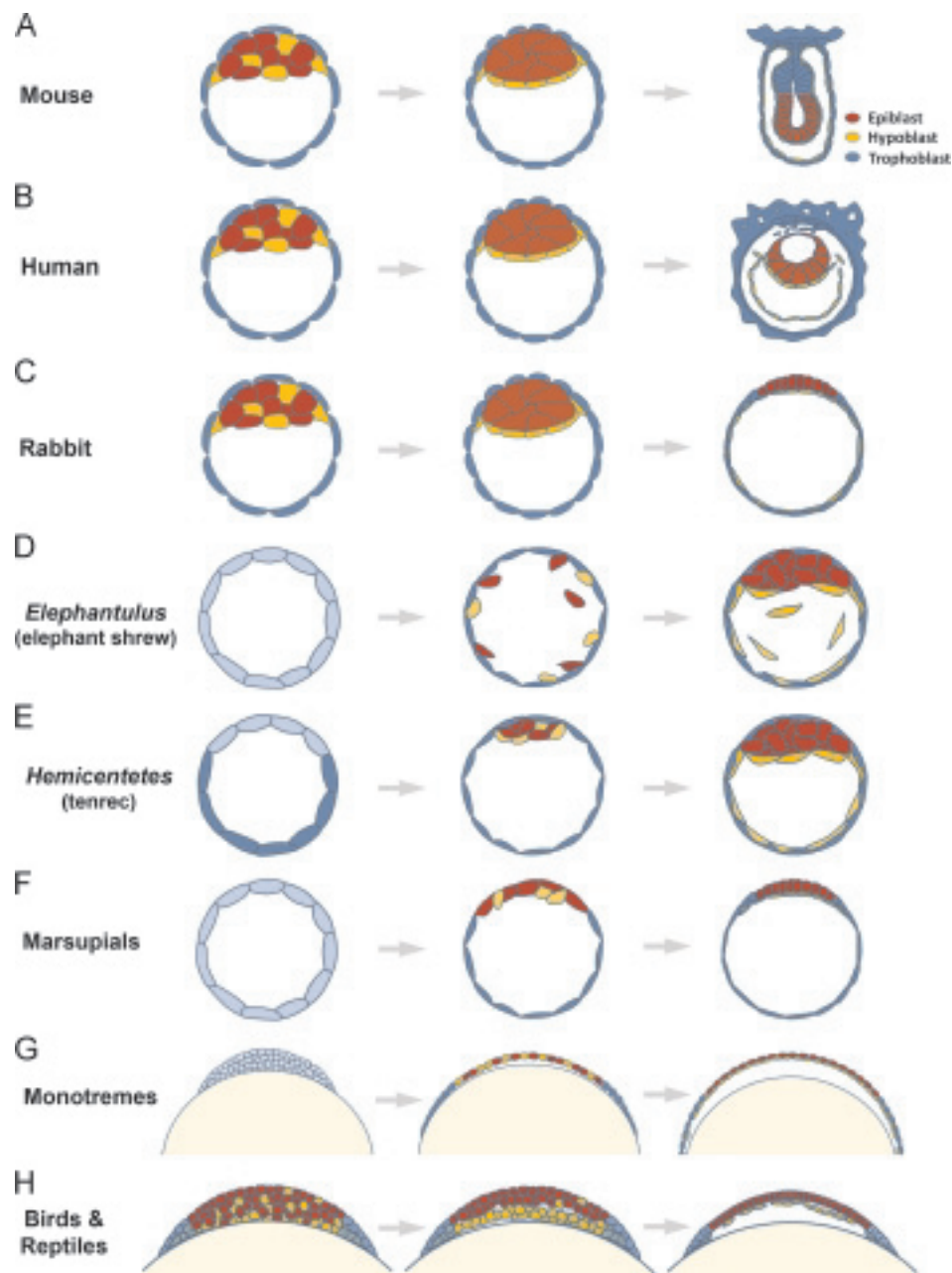


Fig. 1. Morphogenetic diversity in epiblast formation. Representative features of morphogenetic events leading to the formation of the epiblast in several amniote groups. Red: epiblast; Yellow: hypoblast; Blue: trophoblast. (A) Mouse; (B) human; (C) rabbit; (D) *Elephantulus*; (E) *Hemicentetes*; (F) marsupials; (G) monotremes; (H) birds and reptiles. In ((D) and (E)) features after the segregation of the epiblast and hypoblast precursors are omitted.

epithelialization process of the epiblast (Bedzhov and Zernicka-Goetz, 2014). After blastocyst formation, the ICM differentiates into mixed populations of precursor cells for the epiblast and the primitive endoderm (referred to as hypoblast in this review) (Fig. 1A, left). The hypoblast precursors sort out and polarize first to form an epithelialized sheet. The remaining apolar epiblast cells are stimulated to polarize by signals from the basement membrane of two surrounding epithelia (the hypoblast and trophoblast) (Fig. 1A, middle), resulting in the formation of an epiblast rosette with its apical lumen at the center. The lumen expands and forms the rudimentary proamniotic cavity. The epithelialized epiblast expands and fuses with the epithelializing chorionic ectoderm precursors derived from the polar trophoblast, and as a consequence further expanding the proamniotic cavity (Fig. 1A, right). It is worth noting that the border between the epiblast and trophoblast-derived chorionic ectoderm is

not the embryonic–extraembryonic boundary as often depicted, because all of the ectoderm cells in the amnion, which is an extraembryonic structure, come from the epiblast. Furthermore, all mesoderm cells, including those in the amnion, chorion and chorioallantoic placenta, are also epiblast-derived.

Epiblast formation in the human

Based on limited morphological information (Niakan et al., 2012), early human development leading to the segregation of epiblast and hypoblast precursor cells at late blastocyst and peri-implantation stages is very similar to that described in the mouse (Fig. 1B, left and middle). However, molecular data suggest that human-specific variations to the mouse prototype may be more

prominent than previously expected (De Paepe et al., 2012; Kuijk et al., 2012; Niakan and Eggan, 2012). Epithelialization of the epiblast in the human embryo takes place after implantation, and is inaccessible for molecular analysis. Morphological data suggested that the early phase of human epiblast epithelialization is similar to the mouse in that polarization and epithelialization of the epiblast precursor cells result in the formation of a rudimentary proamniotic cavity (Fig. 1B, right) (Luckett, 1975, 1978; Vögler, 1987). Unlike in the mouse, however, this epithelialized epiblast ball loses contact and does not fuse with the chorionic ectoderm. The proamniotic cavity remains as the bona fide apical lumen of the epiblast sheet before and during gastrulation (Fig. 1B, right). Radial symmetry-breaking in the human embryo thus does not involve signals from the epiblast–chorionic ectoderm boundary as known in the mouse embryo. The difference in the mode of epiblast epithelialization and proamniotic cavity formation between these two species accounts for the “inverted” epiblast topology in the mouse embryo and the more “flat”, chick-like one in the human embryo. As in the mouse, all amniotic ectoderm and extraembryonic mesoderm cells in the human are derived from the epithelialized epiblast.

Epiblast formation in the rabbit

In a large number of phylogenetically diverse eutherian species, including the rabbit, pig, cow, sheep, cat and dog (Williams and Biggers, 1990), epiblast precursor cells polarize to form an epithelial sheet in a manner very different from what is known in mouse and human embryos. In these animals, the overall morphological sequence of the initial ICM formation and epiblast/hypoblast precursor segregation takes place like in the mouse and human (Fig. 1C, left and middle) (Degrelle et al., 2005; Oestrup et al., 2009; Vejlsted et al., 2005). But during the process of epiblast epithelialization, their polar trophoblast, the Rauber's layer, degenerates (Betteridge and Flechon, 1988; Blomberg et al., 2008; Flechon et al., 2004; Vejlsted et al., 2005; Williams and Biggers, 1990), and as a consequence the epiblast sheet is exposed directly to the external environment and fuses laterally with the mural trophoblast (Fig. 1C, right). The final organization of the epithelialized epiblast in these animals is comparable to that in the birds/reptiles. Details of this fusion process are poorly understood. For example, it is unclear how the disappearance/apoptosis of the polar trophoblast is coordinated with the polarization of the epiblast cells to ensure the structural (epithelial) integrity of the embryo, or whether the polar trophoblast makes any cellular contribution to either the epiblast or the mural trophoblast. Molecular mechanisms regulating early lineage segregation in these animals also seem to vary. The timing and lineage restriction in the expression of NANOG, OCT4 and CDX2 in the cow were reported to be remarkably different from that in the mouse (Berg et al., 2011; Oestrup et al., 2009; Wolf et al., 2011). The above-mentioned differences and the failure so far in deriving pluripotent stem cells from these animals suggest that caution is needed in extrapolating knowledge in mouse early development to all eutherian mammals (Berg et al., 2011; Gandolfi et al., 2012; Hall and Hyttel, 2014).

Epiblast formation in the elephant shrew and tenrec

The furthest departure in eutherian early development from the mouse prototype is seen in the elephant shrew (*Elephantulus*) and tenrec (*Hemicentetes*) (Goetz, 1939; Van de Horst, 1942, 1949; Wimsatt, 1975). Both of them belong to the Afrotherian clade, the earliest offshoot in the eutherian tree (Romiguier et al., 2013). Early embryogenesis in these two species passes through a hollow “blastocyst” stage at which all cells become part of a “trophoblast”-like

unilaminar epithelium with no ICM (Fig. 1D and E, left). These cells, although polarized and located on the surface of the blastocyst, are not restricted in their fate to the trophoblast lineage because both the epiblast and hypoblast precursors will come from this layer (Fig. 1D and E, middle). In the elephant shrew, the mode of epiblast/hypoblast formation from this unilaminar blastocyst is non-regionalized and the ingression occurs throughout the unilaminar structure. In the tenrec, the ingression takes place preferentially from one pole. As a cell population, the unilaminar blastocyst in the elephant shrew and tenrec resembles the pluriblast (discussed below) in marsupial embryos in that these epithelial, surface-located cells are multipotential. Unlike in the marsupial case, however, the internalized epiblast and hypoblast precursors in the elephant shrew and tenrec aggregate and sort out, eventually reaching an embryonic organization similar to that in the mouse, with the epiblast cells surrounded by the trophoblast and hypoblast cells (Fig. 1D and E, right). These epiblast cells will secondarily epithelialize in a manner akin to what is known in the human (Van de Horst, 1949).

Epiblast formation in marsupials

The marsupials consist of several hundred extant species and form a sister group to the eutherian mammals. Their early development has been studied in a dozen or so species. Post-fertilization cleavage patterns vary, but in all of them a unilaminar, epithelial-like blastocyst stage is reached, and all cells in this unilaminar structure appear indistinguishable (Selwood, 1992) (Fig. 1F, left). Like in the elephant shrew and tenrec, these cells take up the morphology of the trophoblast, but are not restricted fate-wise to this lineage. The precursors for the epiblast and hypoblast, called the pluriblast in marsupial embryos, are restricted in their position to one end of this unilaminar structure. The inside-outside model for the segregation of the ICM and trophoblast cell lineages is therefore not applicable to the marsupial embryos. Nevertheless, it has been argued that a certain degree of ultrastructural/morphological heterogeneity can be discerned among the epithelial blastocyst cells (Selwood, 1992). This may have a causal link to asymmetric cell divisions during early cleavages (asymmetric inheritance of maternal determinants) and to a bias later on in their fate choices (trophoblast vs. pluriblast). Molecular data from the opossum support this model (Morrison et al., 2013), whereas data from the tammar wallaby argue against it (Frankenberg et al., 2013). By the late unilaminar and early bilaminar stages, heterogeneity in blastocyst cell morphology becomes more prominent. The pluriblast cells now take up cuboidal or columnar epithelial morphology and the hypoblast precursors delaminate from the pluriblast region of the blastocyst (Fig. 1F, middle). The internalized hypoblast cells spread and migrate to cover the under-surface of both the epiblast (the remainder of the pluriblast after hypoblast delamination) and the trophoblast (the non-delaminating part of the unilaminar blastocyst), thus generating a prototypic two-layered structure with three well-defined cell lineages (Fig. 1F, right). The epiblast cells in marsupial embryos are considered to have polarized, epithelial morphology from the very beginning, even prior to their specification as the epiblast precursor cells.

Epiblast formation in monotremes

The monotremes (prototherians) have an oocyte organization very similar to that of the reptiles and birds (Flynn and Hill, 1939). They represent an early branch in mammalian evolution. Early-stage monotreme embryos are difficult to obtain and our knowledge of their early development came primarily from three papers published by Hill and colleagues (Flynn and Hill, 1939, 1947; Wilson and Hill, 1908), the last of which investigated the developmental

period leading to the formation of a bilaminar structure in *Echidna* and the platypus and is summarized below. Up to the multilayered blastoderm stage (Fig. 1G, left), monotreme early development resembles that of the reptiles and birds (Bertocchini et al., 2013; Flynn and Hill, 1947; Sheng, 2014). A reduction in the cell layer number, likely through radial intercalation, results in the formation of a unilaminar, “blastocyst”-like structure (Flynn and Hill, 1947; Selwood and Johnson, 2006) (Fig. 1G, middle). This “blastocyst” is incomplete since the epiboly movement (the process of blastoderm cells migrating from the embryonic to the abembryonic side and eventually enclosing the entire yolk) at this point has brought the blastoderm edge cells only to the yolk equator. As the epiboly continues and the entire yolk is being covered by a unilaminar epithelium, cells located in the central area of this unilaminar structure, above the original germinal disc, are presumably specified as precursors for the epiblast and hypoblast, and the peripheral cells become precursors for the trophoblast (Flynn and Hill, 1947; Selwood and Johnson, 2006). It is unclear whether the central cells are bi-potential for both the epiblast and hypoblast fates, as the equivalent of the eutherian ICM or the marsupial pluriblast, or are a salt-and-pepper mixture of pre-biased/pre-determined cell populations as suggested from morphological heterogeneity observed among these cells (Flynn and Hill, 1947; Selwood and Johnson, 2006). Formation of a bilaminar structure follows a similar process as described in the marsupial embryo. The hypoblast precursor cells delaminate from the central area of this unilaminar blastocyst and subsequently spread underneath the epiblast and trophoblast (Fig. 1G, right). Selwood and Johnson (2006) had proposed that segregation of the epiblast and hypoblast precursors in monotreme embryos takes place even before the blastoderm achieves a unilaminar structure.

Epiblast formation in birds and reptiles

The sauropsids (birds and reptiles) are an important branch of Amniota. Morphogenetic events leading to the separation of the trophoblast, epiblast and hypoblast lineages in a eutherian embryo are thought to have an evolutionary origin in the ancestral mammal with an avian/reptilian-like developmental program. The birds and reptiles are sometimes referred to as two separate groups, but phylogenetic evidence strongly suggest that the avian clade is a part of the reptilian tree, with the crocodiles being its closest living relatives (Chiappe, 2004; Chiari et al., 2012). Research on avian and reptilian early development has a long history (Bertocchini et al., 2013; Sheng, 2014), but molecular investigation has been limited primarily to one avian species, the chicken (*Gallus gallus*). In a freshly-laid chicken egg, embryonic development has reached EGK-X (Eyal-Giladi and Kochav, 1976), a stage at which three main cell lineages have already been delineated: the epiblast, hypoblast and area opaca cells (Alev et al., 2013a; Lavial et al., 2007; Pernaute et al., 2010; Shin et al., 2011; Yatskievych et al., 1999) (Fig. 1H, right). These lineages are broadly equivalent to the epiblast, hypoblast and trophoblast, respectively, in a mammal embryo. A notable difference is that lower layer cells in the area opaca, located underneath the trophoblast-like extraembryonic ectoderm and akin to the mammalian parietal primitive endoderm cells in their function, are not derived from the hypoblast in the chick. Morphogenetic events leading to the separation of these three lineages take place intrauterally and have not been studied at the molecular level. The model presented here (Fig. 1H, left and middle) is based on our unpublished data on intrauterine chick development and on epiblast epithelialization and lineage segregation processes in pre-gastrulation chick and zebra finch embryos. The first half of the intrauterine development in the chick embryo results in the

formation of a multi-layered blastoderm (Fig. 1H, left), which during the second half is gradually reduced to a two-layered structure through shedding and radial intercalation (Fig. 1H, middle, right). Molecular specification of the three primary lineages accompanies this cellular rearrangement. Data from zebra finch embryos suggest that lineage markers are already expressed in a salt-and-pepper manner at the beginning of the layer reduction phase (Fig. 1H, left), with epiblast markers being gradually restricted to the upper layers and hypoblast markers to the low ones (Fig. 1H, middle), eventually leading to the two layered epiblast-hypoblast organization with distinct molecular signatures (Fig. 1H, right). This layer-reduction process is similar to that described in monotreme embryos (Flynn and Hill, 1947; Selwood and Johnson, 2006) and our molecular data appear to support the pre-determination model proposed by Selwood and Johnson (2006). An important distinction between the layer-reduction processes in the birds/reptiles on one hand and the monotremes on the other is that in the former the blastoderm never reaches a unilaminar stage and juxtaposition of the epiblast and hypoblast precursors within a single epithelialized layer is minimal.

A model for the amniote prototype

Based on the above discussion, two prototypic views of epiblast epithelialization are presented in Fig. 2, one for the amniotes in general and the other for the eutherian mammals in particular. The ancestral amniote is considered to have an egg organization and early embryonic development similar to what is known in extant sauropsids and prototherians (Fig. 2A). Early cleavages are meroblastic, with cytokinesis lagging behind nuclear divisions. This is followed by the cellularization process and by a phase of rapid cell divisions, resulting in the formation of a multi-layered blastoderm with dome-shaped and presumably totipotent “blastomeres” positioned on top of a giant yolk cell. After blastomere layer reduction and the epiboly movement, the embryo reaches a 1- or 2-cell-layer thick “blastocyst” stage. Specification of the trophoblast, epiblast and hypoblast lineages takes place concomitant with this phase of cellular rearrangement. These two processes are presumed to be under separate molecular regulation. If layer reduction (radial intercalation) advances more rapidly than fate specification, a unilaminar blastoderm, as seen in monotreme embryos, is reached. In such a case, the central cells become epiblast/hypoblast precursors either as a bi-potential cell population (similar to the pluriblast in marsupials) or a mixture of uni-potential cells, and the peripheral ones become the trophoblast. Hypoblast cells secondarily move out from this “pluriblast” layer, resulting in the prototypic two-layered epiblast-hypoblast organization. If the layer reduction process lags behind the fate specification process, as seen in bird/reptile embryos, the epiblast and hypoblast cells are specified and sort out into two layers before the embryo ever reaches a unilaminar stage. In both scenarios, the relationship between the trophoblast and the pluriblast is that of peripheral vs. central, instead of outside vs. inside (Johnson and Selwood, 1996). The epiblast as an epithelium is therefore topographically continuous with the trophoblast epithelium.

A model for the eutherian prototype

In ancestral eutherian mammals, the amniote prototype is modified to accommodate two types of physiological changes: yolk reduction and placentation. Early divisions in ancestral eutherians are holoblastic and a unilaminar blastocyst stage is reached without

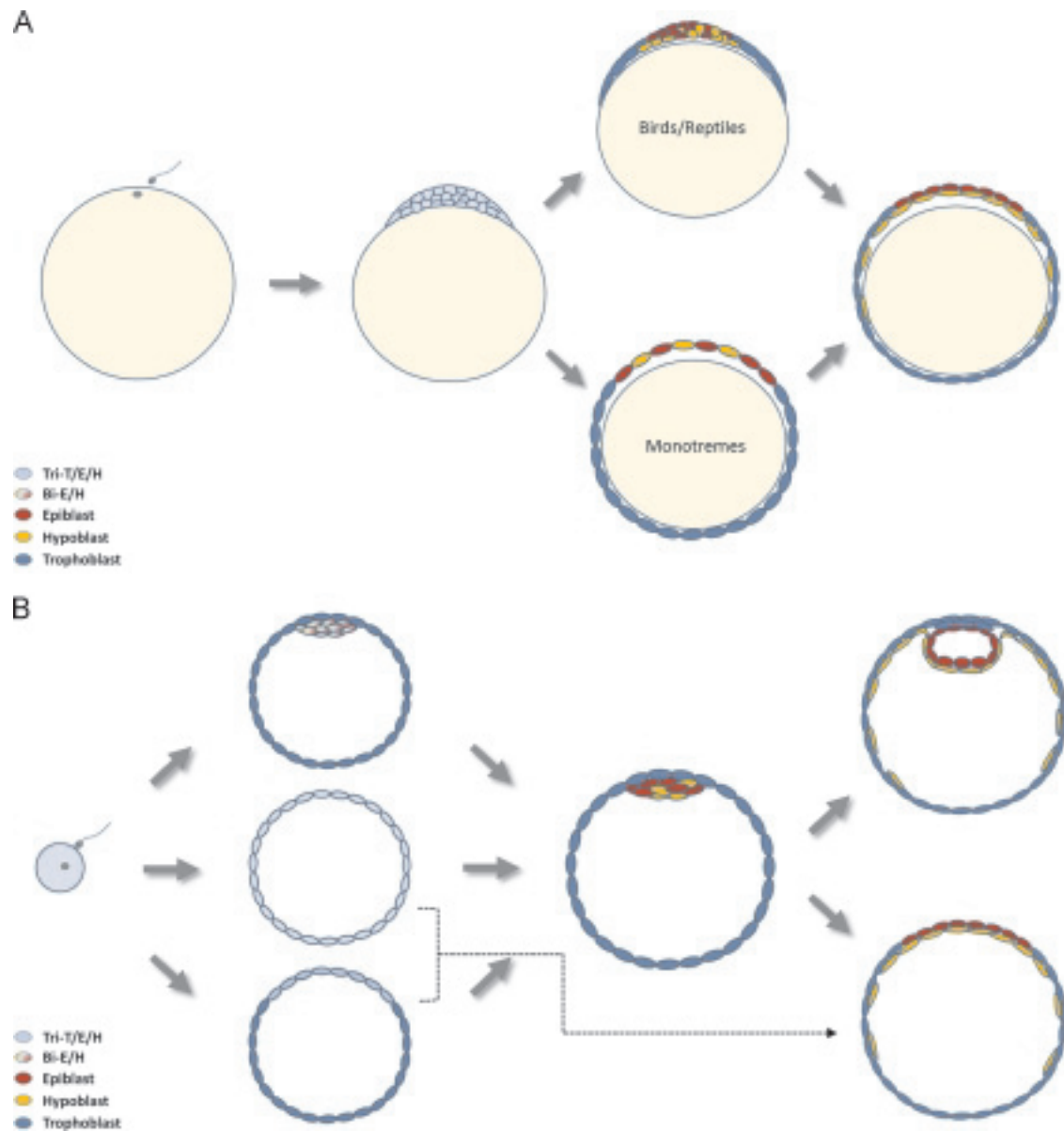


Fig. 2. Models for the amniote and eutherian prototypes of epiblast epithelialization. (A) The amniote prototype. (B) The eutherian prototype. Red: epiblast; Yellow: hypoblast; Blue: trophoblast; tri-potential (T/E/H) or bi-potential (E/H) cells are also color-coded as indicated. In (A) represented by the reptilian, avian and monotreme groups, the mature oocyte is yolky and early cleavages meroblastic. Lineage segregation and blastomere layer reduction are hypothesized to be under separate regulation. If layer reduction outpaces lineage specification (as seen in monotreme embryos, bottom route), a unilaminar blastocyst stage is reached. This is followed by delamination of hypoblast precursors. Otherwise, the bilaminar epiblast–hypoblast organization is reached without going through a unilaminar blastocyst stage (as seen in bird/reptile embryos; top route). In (B) represented by all eutherian mammals, the oocyte contains little or no yolk. Cleavages are holoblastic. A unilaminar blastocyst stage, containing all (middle route) or partial (bottom route) tri-potential cells, is reached. Both the epiblast and hypoblast precursors delaminate from this unilaminar structure and aggregate to form the mixed epiblast and hypoblast cell mass, which after two rounds of epithelialization forms the epiblast and hypoblast epithelia. Pre-mature differentiation of the trophoblast in some eutherians (top route) may have facilitated the formation of the ICM. An ICM also ultimately forms in the other two routes. Depending on the implantation mode, the epithelialized epiblast may be covered by the polar trophoblast (top right) or be in continuity with the mural trophoblast (bottom right). The dotted line represents the marsupial prototype, possibly by going through a bi-potential pluriblast stage.

undergoing the epiboly process (Fig. 2B), Marsupials represent an evolutionary intermediate and the elephant shrew and tenrec represent the early eutherian prototype. All cells of the unilaminar blastocyst are epithelial and totipotent at this stage. Division into the trophoblast and pluriblast areas may be decided stochastically or be influenced by asymmetric signals associated with implantation or inherent structural asymmetry. Early specification of the trophoblast lineage as seen in the mouse and human is likely a consequence of early implantation in these species. If the embryonic side of the blastocyst is involved in the early implantation, a

layer of polar trophoblast is evolved to cover the pluriblast (the ICM) population. Although the origin of the polar trophoblast and its relationship to the pluriblast (the ICM) have not been clearly resolved, in all eutherian species a conserved developmental stage is eventually reached, with a well-differentiated trophoblast layer (including the mural and polar trophoblast) and an ICM consisting of mixed epiblast and hypoblast precursor populations (Fig. 2B). In contrast, marsupial embryos never pass through this stage, suggesting that acquisition of the polar trophoblast fate was a eutherian invention, likely related to the mode of implantation in its

ancestors. In many extant eutherian species in which the embryonic side is no longer actively involved in the establishment of fetal–maternal contact, the polar trophoblast is secondarily lost, resulting in an epithelialized epiblast which is topographically continuous with the mural trophoblast as known in the sauropsids, monotremes and marsupials.

Epiblast formation as an evolutionary adaptation to functional constraints

The epiblast as a cell lineage is defined by its unique molecular signature and its functional contribution to the three germ layers (Fig. 3A). The significance of the epiblast as an epithelial structure is

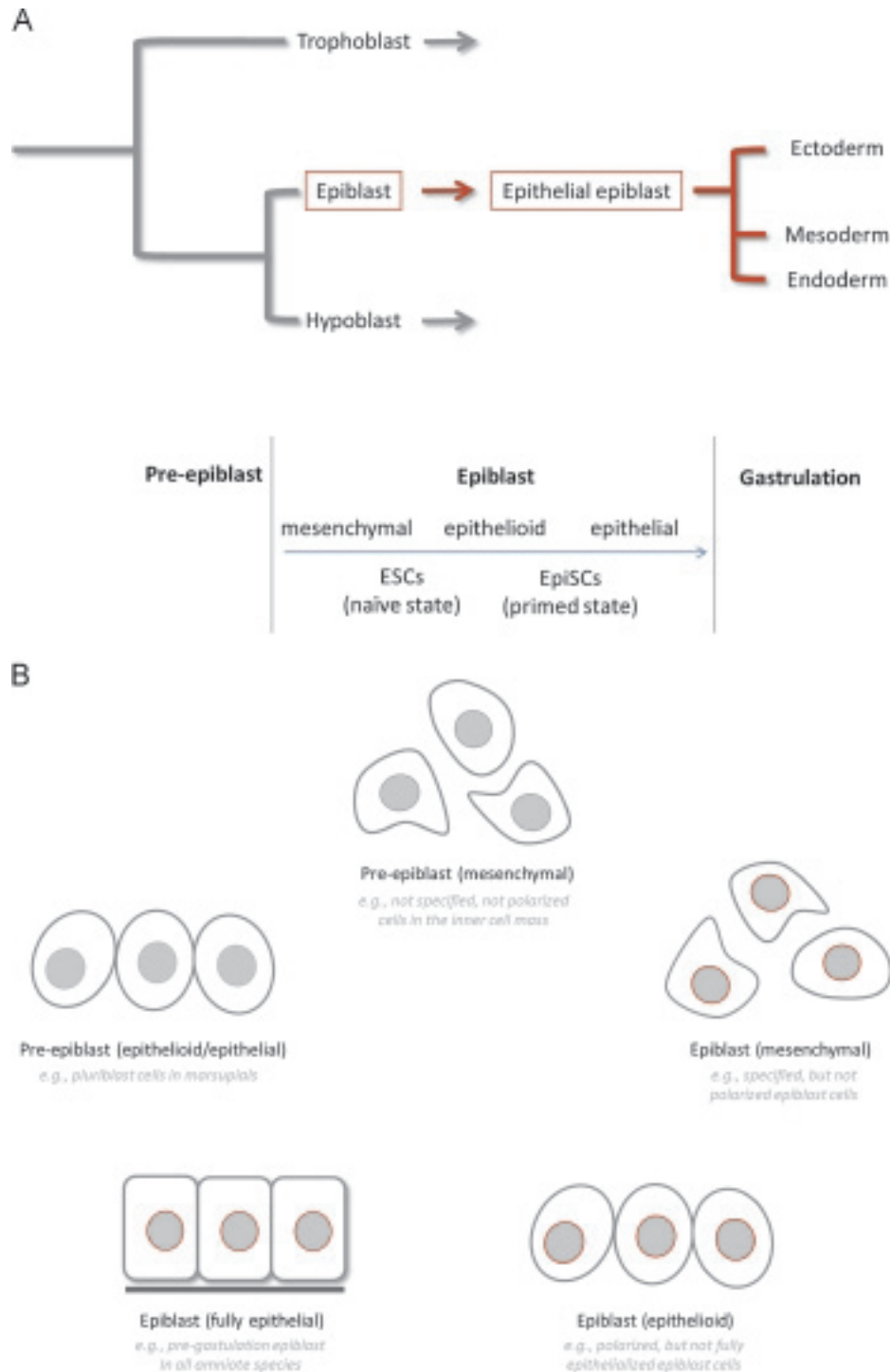


Fig. 3. Relationship between form and function in epiblast morphogenesis. Form is the cell morphology and function is the cell lineage/fate. The epiblast lineage is defined by molecular criteria and the epithelial epiblast is defined by cell biological criteria. Pre-epiblast indicates tri-potential or bi-potential precursor stage. (A) Lineage segregation in the mouse embryo. After molecular specification, the epiblast cells undergo morphological changes from mesenchymal to epithelial before imitating the gastrulation process. The hypothetical relationship between the epithelialization status and the type of pluripotent cells is also indicated. (B) Epiblast or pre-epiblast (tri-potential or bi-potential precursor) cells in amniote embryos can exist in either mesenchymal or epithelial state. A fully epithelialized epiblast is the final state in all amniotes. How to reach this final state varies from species to species. For example, in the mouse embryo, the sequence is mesenchymal pre-epiblast/mesenchymal epiblast/epithelioid epiblast/fully epithelial epiblast. In stead, the sequence in marsupial embryos is epithelioid pre-epiblast/epithelioid epiblast/fully epithelial epiblast.

not clear. In vivo, all intraembryonic tissues are generated from the epiblast through gastrulation, the starting point of which is the unilaminar, fully-epithelialized epiblast. This requirement, however, is specific to the amniotes because in most amphibian and fish species the pre-gastrulation ectoderm is multi-cell layer thick and not as well-epithelialized as the epiblast (Nakaya and Sheng, 2008). Germ layer differentiation per se, for example from the pluripotent amniote ES or iPS cells, does not need to start from an epithelialized structure (Pieters and van Roy, 2014). The conservation of an epithelialized epiblast in all extant amniotes suggests that their common ancestor was under selection pressure to maintain the pre-gastrulation ectoderm as a full epithelium to counteract mechanical stress unique to the amniote early development. Such a requirement for epithelial integrity is likely associated with novel morphogenetic features in the amniotes, including the need to generate extraembryonic tissues (e.g., the amnion and the chorion) and the shift from a circumblastoporal mode of gastrulation to a posterior-epiblast restricted one (Alev et al., 2013b; Bertocchini et al., 2013). These features necessitate the subdivision of the ectoderm into embryonic and extraembryonic regions, the uncoupling of the gastrulation and epiboly processes, and finally the full epithelialization of the epiblast.

Epiblast formation offers a unique window for capturing the pluripotent state of pre-gastrulation ectoderm

While many genes involved in pluripotency maintenance and reprogramming, e.g., NANOG (Scerbo et al., 2014) and class V POU domain proteins (Frankenberg and Renfree, 2013), are molecularly and functionally conserved and can trace their evolutionary origin to at least the common ancestor of the osteichthyans (bony fish and tetrapods), stable in vitro culture of pluripotent cells derived from pre-gastrula stage embryos or through reprogramming remains very inefficient in most species. Pre-gastrulation ectoderm cells by default are pluripotent because they are capable of giving rise to all cell types in the three germ layers after gastrulation. The pluripotency of these pre-gastrulation ectoderm cells, however, is maintained only for a brief period of time during normal development. Capturing such a narrow developmental window in artificial culture conditions requires proper understanding of how pre-gastrulation ectoderm cells regulate the timing of lineage differentiation (e.g., as an evolutionarily conserved role of POU-V proteins (Tapia et al., 2012)) in coordination with other morphogenetic events that are not directly related to pluripotency maintenance (e.g., overlapping roles of signaling pathways in regulating pluripotency and morphogenesis (Sokol, 2011)). The amniote epiblast from such a perspective can be viewed to go through divergent morphogenetic processes to reach a final cell biological status that is conserved among all amniotes, but distinct from that in other vertebrate groups. Interestingly, epithelialization of the amniote epiblast may confer a certain advantage for efficient capture of such a transitory, pluripotent stage in vitro. It has been shown that optimal reprogramming of somatic cells into induced pluripotent cells requires a mesenchymal-to-epithelial transition process (Esteban et al., 2012), and that epithelial adhesion protein E-cadherin mediates LIF-dependent self-renewal in mouse ES cells (Bedzhov et al., 2013; del Valle et al., 2013; Pieters and van Roy, 2014). Although mouse ES cells tend to form aggregates of tightly adhering cells without clear epithelial morphology, both mouse epiblast-derived stem cells (EpiSCs) and human ESCs form colonies reminiscent of an epithelialized epiblast. Recent transcriptional profiling data suggested that both mouse ESCs and mouse EpiSCs capture transitory developmental status of the epiblast, albeit at different stages of epiblast maturation (Boroviak et al., 2014) (Fig. 3A). These lines of evidence suggest that the process of epiblast epithelialization in vivo may provide a unique cell biological window for capturing pluripotent stages of pre-gastrulation ectoderm in vitro,

with optimal “culturable” stages and culture conditions being variable in different amniote species due to variations in the molecular and cellular regulation of epiblast morphogenesis in vivo.

Epiblast formation in light of the evolutionary hourglass model

Using the analogy of the hourglass model, in which a pharyngula (a general term used to describe an embryonic stage after the neurula, with conserved branchial arches and pharyngeal pouches) is the phylotypic stage of all vertebrate embryos, one may consider that amniote embryogenesis shares the earliest phylotypic stage with two conserved features: the specification of the three primary cell lineages (trophoblast-like extraembryonic ectoderm, epiblast and hypoblast) and the epithelialization of the pre-gastrulation epiblast. Development prior to the appearance of these two features is not conserved and each group uses different morphogenetic strategy to reach this conserved phylotypic stage (Fig. 3B). Commonly-held notions about how the three lineages separate step-wise from each other and how these steps are manifested morphologically should be considered in light of this morphogenetic diversification. With respect to mammalian early development, three such notions, listed below, deserve re-evaluation. (1) The first step, the segregation between the trophoblast and the ICM (bi-potential precursors of the epiblast and hypoblast), is dictated by positional cues. Blastomeres located superficially will become trophoblast cells and those located internally will become ICM cells. (2) The trophoblast as an epithelium enclosing the blastocyst is the first lineage to be specified and after its specification the trophoblast does not make any contribution to the other two lineages. (3) From the early blastocyst stage the ICM is completely sealed off by polar trophoblast cells and the cellular and molecular distinction between these two cell populations can be clearly made. These notions, obtained primarily from studies of the mouse embryo, cannot be applied to the amniotes in general as we have discussed in this review. Neither can they be viewed as representing all mammals or all eutherian mammals. Most importantly, an appreciation of morphogenetic diversity in epiblast formation may help us understand many subtle differences observed in mammalian early lineage specification which cannot be easily explained with current prevailing models, including for instance the fact that trophoblast cells in the cow maintain the ICM marker OCT4 expression up until the late blastocyst stage (Berg et al., 2011), that human trophoblast cells express pluripotency markers and maintain the capacity to differentiate into the ICM lineage (De Paepe et al., 2012), and that blastomeres located inside the blastocyst may initiate trophoblast differentiation in the absence of maternal and zygotic E-cadherin (Stephenson et al., 2010).

Summary

The epiblast is one of the three primary lineages specified early during amniote development. An epithelialized epiblast is evolutionarily conserved in the amniotes. It is the starting material for gastrulation through which it gives rise to all intraembryonic tissues. Morphogenetic processes leading to its formation vary substantially in different amniote groups. Within the eutherian group, obvious departure from what is known in the mouse model is noted. Subtler differences in epiblast morphogenesis and lineage specification between the mouse and the human may be understood more easily in light of such diversity. In reptiles, birds and eutherian mammals, epiblast cells are not organized as an epithelium when they are first specified molecularly. An MET process has to take place to reorganize these cells into a unilaminar structure

with all the essential features of a fully-formed epithelium (Fig. 3B). Understanding the cell biology of this MET may ultimately help us achieve optimal conditions to maintain pluripotent epiblast cells in culture.

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